domain-like (Ig-) region. Using sedimentation-velocity centrifugation and size-exclusion chromatography of purified human titin fragments, we detected a monomeric state of the titin N2B-domain or the N2B-unique sequence contained therein. The constitutively expressed PEVK-domain and the Ig-only fragment I9-I12 also behaved as monomers in-vitro, whereas the N2A-segment showed both dimeric and (to a lesser degree) monomeric behavior. Yeast-2-hybrid and GST-pulldown interaction tests demonstrated that both the N2B and the N2A domain, but not the PEVK-fragment, bind to the small heat-shock proteins (sHSPs), alphaB-crystallin and HSP27. These interactions were confirmed on isolated human cardiac or rabbit psoas myofibrils incubated ex-vivo with either sHSP type. Stretching myofibrils to promote titin-domain unfolding increased the propensity of sHSPs to bind I-band titin. Protein unfolding in-vitro by 8M urea caused aggregation of the N2A-segment under acidic conditions (pH6.7), but not at pH7.2. Importantly, alphaB-crystallin prevented the aggregation of the N2A-segment at pH6.7 partially (molar ratio of N2A:alphaB-crystallin, 1:5) or fully (ratio, 1:10). In cultured neonatal rat cardiomyocytes, both sHSPs translocated from the cytosol to the sarcomeric Z-disk/I-band region on inhibition of the proteasome. In adult rat cardiomyocytes both sHSPs associated with I-band titin already under normal culture conditions, suggesting an age-related increase in chaperoning activity. We conclude that titin filaments may run through the elastic segment mainly as monomers; sHSPs associate with elastic titin domains under various stress conditions; and sHSPs are able to protect I-band titin regions from aggregation under adverse intracellular circumstances.

**1824-Pos Board B594**

**Z-Line Elongation Observed in Titin Labeled Myofibrils**

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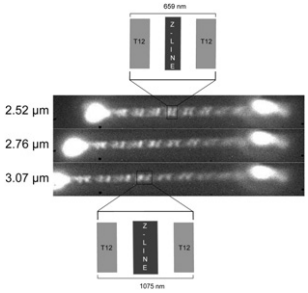
A muscle myofibril (below) is composed of sarcomeres in series. These sarcomeres are made of myosin, actin and titin, representing the three major filamentous proteins in muscle. Titin links myosin in the A-band region, to actin 100nm from the Z-line. This particular junction where titin binds actin, is where T12 antibody localizes. Using single myofibrils isolated from rabbit psoas muscle, we labeled titin with T12 conjugated to quantum dots and stretched myofibrils between a cantilever and rigid glass needle. Preliminary data suggests that myofibrils display a broadening between these T12 bands when stretched, despite being considered mainly inelastic due to titin-actin interactions. Interestingly, densitometric analysis revealed the distance between flanking T12 bands increased, while the width of the fluorescent bands remained unchanged. Two alternative explanations could require the width of the Z-line to increase with stretch (Tonino et al., 2009), or titin could dislodge from actin to accommodate stretch. Given the longest average sarcomere length was 3.07µm, the actin dislodging seems less likely as the psoas muscle has not approached peak tension (~3.9µm (Wang et al., 1991)). Thus, it appears that Z-line width increases with stretch to a greater extent than previously reported.




**1825-Pos Board B595**

**Half-Sarcomeres with Heterogeneous Crossbridge Populations can be Stabilized by Accompanying Variations in Titin Filaments**

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Residual force enhancement occurs when the steady-state force produced by a contracting muscle is increased following stretch even though the average actin-myosin overlap within sarcomeres is reduced. We recently used a computational model to examine the mechanical properties of a system of serially-connected half-sarcomeres. The simulations demonstrated that such a model exhibits force enhancement when the active force generating capacity of the individual half-sarcomeres (the number of functioning myosin heads) varies by as little as 2% (Campbell et al., PLoS Comput Biol 2011). Variability at this level would be hard to detect in real experiments but is difficult to discount in biological systems. We have now performed additional simulations in which we vary the passive mechanical properties of the individual half-sarcomeres (the non-linear stiffness of their titin filaments) as well as their active mechanics. The results show that when both active and passive properties are varied, lengths of half-sarcomeres prior to stretch do not always correlate with post-stretch values. This finding may explain experimental observations of 'stronger' (shorter) half-sarcomeres prior to stretch that yield to a greater degree than 'weaker' (longer) half-sarcomeres. Data published by other groups (Labeit et al., PNAS 2003; Bagni et al., Biophys J 2002) suggest that the stiffness of titin molecules increases with the intracellular $Ca^{2+}$ concentration. We predict that when we add $Ca^{2+}$-dependent titin stiffness to our model it will further stabilize half sarcomeres after stretch (reduce half-sarcomere length variability). This might help to prevent stretch-induced damage, thus increasing the resiliency of the contractile system. A computational model that combines active and passive half-sarcomere variability with a $Ca^{2+}$-activated stiffness may therefore offer a more complete explanation of residual force enhancement and related phenomena.

**1826-Pos Board B596**

**Hysteresis and Efficiency in Passive Skeletal Muscle Myofibrils**

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Determining mechanical properties of titin is difficult but the passive properties of single myofibrils are mostly attributed to titin. We investigated whether myofibril behaviour mirrors that of single titin molecules. Single myofibrils were subjected to three passive stretch-shortening cycles of up to 3.5µm/sarcomere at a speed of 0.1 µm/sarcomere/second.

Stretched-shortened myofibrils (Figure 1) show reduced force during stretch for a given sarcomere length (SL) and reduced peak force for cycles 2 and 3 compared to cycle 1. Force-SL curves for all 3 cycles during the shortening are similar. We see increased efficiency (shortening energy/lengthening energy) with repeated cycles (37%, 48% and 53%). These properties are in general agreement with results observed in single titin preparations (Kellermayer et al., 1997). Titin properties can be studied using single myofibrils. In the future, we would like to study titin properties in calcium activated myofibrils in which active (actin-myosin based crossbridges forces) are eliminated either by chemical inhibition or by deletion of regulatory proteins on actin.

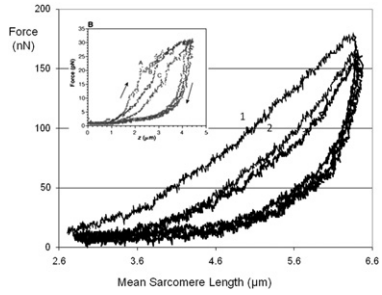


Figure 1. Force and mean sarcomere length for a myofibril subjected to 3 sequential stretch-shortening cycles. Insert shows hysteresis behaviour of a single titin molecule (Kellermayer et al., 1997; *Science:*276-1112-1116).

**1827-Pos Board B597**

**Removal of Ig Domains of Titin Alters Contractility in Mouse Soleus Muscle**

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The giant protein titin provides elasticity to the skeletal muscle sarcomere by acting as a molecular spring. We created a mouse model in which 9 immunoglobulin (Ig) domains (Ig3-11) have been deleted from titin's spring region, providing a novel model to test the effect of a shorter spring on the physiology of skeletal muscle. The soleus muscle was studied by using gel electrophoresis and functional measurements on contractility made via an intact muscle mechanics system on six month old male mice. An additional set of mice were exposed to exercise training on a treadmill to determine exercise capacity and response to exercise. Soleus mass was lower in the IGKO ($8.3 \pm 0.2$mg) vs WT ($10.2 \pm 1.2$mg). IGKO mice showed a significant decrease in titin size on 1% agarose gels greater than the 88KDa loss predicted by the deletion if Ig3-11. Furthermore, IGKO mice have a reduced myosin II/ myosin I ratio (2.1 vs. 8.0, p=0.002). Intact muscle mechanics measurements probed both passive and active tension. IG KO muscles produced an increased passive tension (19% p=0.02) at 20% above slack length of the muscle. Active force measurements probed at the optimal twitch length (Lo) revealed a significantly decreased twitch/tetanus ratio in IGKO's (12.4% vs 8.1%, $p<0.001$) and decreased active force at all frequencies examined (1-150Hz). IGKO also experienced less fatigue (28% vs 67% decrease, p=0.01). Exercise testing in mice showed a significantly reduced maximum running speed between IGKO and WT (18.8 vs 22.0 m/min, p=0.005), which persisted after two weeks of training. The IgKO mouse provides a unique model for elucidating the effect of titin's spring elements on contractility in skeletal muscle.